学位論文の要約

学位論文題目 Evolutionary developmental studies on the role of hormone receptor signaling during termite soldier differentiation
（シロアリの兵隊分化におけるホルモン受容体シグナリングに関する進化発生学的研究）

地球生命環境科学専攻

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Morphological and ecological novelties drive organismal evolution and diversification via opening up new adaptive zone. Molecular mechanisms underlying evolutionary novelties have been analyzed in many organisms. It has been proposed that changing of recruitment or co-option of existing gene network generates evolutionary novelty, due to little or no changes of genes, such as cis-regulatory related evolution of novelties rather than arising new genetic mechanisms. I focused on the soldier caste as evolutionary novelty that might be the trigger of social evolution and diversification of termites within the cockroach clade. I hypothesized that the co-option or recruitment of hormone (juvenile hormone (JH) and 20-hydroxyecdysone (20E)) receptor signaling might be quite important for soldier differentiation, and such a novel function for hormone signalings might drive the acquisition of soldier caste during the course of termite evolution. To clarify this hypothesis, I investigated the roles of JH and 20E hormone receptor signalings and a crosstalk of these signalings for soldier differentiation, and performed the comparative analysis of their roles among termites and cockroaches including the sister group *Cryptocercus* woodroaches.

In Part I, to investigate the role of JH signalings during soldier differentiation, I performed functional analysis of JH receptor signaling genes using RNA interference (RNAi) in the archotermopsid termite *Zootermopsis nevadensis* and closely related cockroaches. In Chapter 1, I found that highly expression levels of JH receptor gene, *Met*, just after the presoldier molt to form soldier-specific morphogenesis such as the mandibular elongation. In Chapter 2, I found that the woodroach *C. punctulatus* possessed the ability of nymphal molt induced by the JHA application. These results showed that the JH-inducible process of molting (plus specific morphogenesis in termites) could be observed in *Z. nevadensis* and *C. punctulatus*. Knockdown of *Met* affected expression levels of ecdysone synthesis genes in both species. Expression patterns of 20E signaling genes after *Met* RNAi, however, were notably different between *Z. nevadensis* and *C. punctulatus*.

In Part II, I focused on the cuticular formation to understand the role of 20E for soldier-specific morphogenesis. In Chapter 3, I found that the expression of tyrosine metabolic genes, especially highly expression levels of *Lac2*, was crusial for soldier-specific cuticular formation in the rhinotermitid termite *Reticulitermes speratus*. In Chapter 4, these functions of tyrosine metabolic genes for cuticular formation of not only soldiers but also other castes were confirmed by RNAi experiments in *Z. nevadensis*. In the presoldier molt, highly expression of

aaNAT gene, involved in the formation of colorless cuticle in other insects, was observed. On the other hand, all of the other tyrosine metabolic genes were basically highly expressed in the soldier molt. Then, in Chapter 5, based on the expression and function analysis in *Z. nevadensis*, it was shown that 20E receptor signaling was crusial for soldier-specific cuticular formation via the regulation of tyrosine metabolic gene expression. Moreover, I found that different expression timing of *EcR* was respectively involved in the regulation of molting process or soldier-specific cuticular formation.

Finally, in Part III, I performed RNA-seq analysis during soldier differentiation and worker molting process under natural conditions in *Z. nevadensis*. I found that most genes showed similar expression pattern during each molting process in spite of their huge phenotypic differences. On the other hand, there was a possibility that some highly expressed genes especially in heads were related to the caste determination during soldier differentiation. Based on the functional analysis of these genes, I concluded that the TGFβ signaling was one of the most crucial pathway for a crosstalk between JH and 20E signalings required for soldier differentiation.

Recent studies proposed a general manner of molecular mechanisms underlying evolutionary novelty. Namely, co-option or recruitment of some important genes and gene networks might be involved in the evolution of some morphological novelties. Based on the results obtained above, I propose the following new scenario for molecular mechanisms of soldier evolution. First, the JH-inducible molting ability might be acquired in the common ancestor of termites and woodroaches. Second, recruitment or co-option of JH receptor signaling with the modification of downstream pathway, for example molecular relationships between JH and 20E signaling genes, might be required for soldier differentiation. Finally, the TGFβ signaling pathway is one of the most important these candidates involved in the signaling activity between JH and 20E. To clearly understand the downstream termite-specific pathway, further analysis on the JH-sensitive genes in termites and woodroaches should be needed. Comparative transcriptomics using NGS technology, e.g. transcriptome analysis under the JH application in termites and woodroaches, and chromatin immunoprecipitation seq (ChIP-seq) analysis of JH receptor in termites, will be effective experimental method. These comparative genomic and physiological analyses will elucidate the evolutionary background of monophyletic group of termites in the cockroach lineage.